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#29

PATENT

Attorney Docket No. 16243-1-5

MAIL ROOM AUG 22 1994 PAT. & TRADEMARK OFC.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Richard H. Tullis	Examiner: J. Martinell
Serial No.: 08/078,767	Art Unit: 1805
Filed: June 16, 1993	<u>DECLARATION PURSUANT TO 37 C.F.R. § 1.132</u>
For: OLIGONUCLEOTIDE THERAPEUTIC AGENT AND METHODS OF MAKING SAME	

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Dr. Dennis E. Schwartz, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. The Exhibits (1-9) attached hereto are incorporated herein by reference.

2. I received a Ph.D. in chemistry and biological science from the Purdue University in 1972. I spent 10 years at Harvard University as a Postdoctoral fellow in the laboratories of Drs. Walter Gilbert (Nobel prize laureate) and Paul Zamecnik (member of the National Academy of Science). My research specialty involves nucleic acid chemistry and biology.

A copy of my curriculum vitae is attached as Exhibit 2.

3. I have been a scientific founder of two companies, MicroProbe Corporation and Origen. I am presently employed by Origen as a founder and am responsible for development of novel therapeutic compounds for human diseases.

Considered 11/21/94

4. I have read and am familiar with the contents of the application and related papers. I understand that the Examiner has made two rejections. The first rejection is for failure to fully teach how to make and use the invention as claimed, and the second is that the invention is obvious over a combination of four references. This declaration will address both rejections. I will provide objective evidence of the state of the art of arresting specific protein expression by oligonucleotide hybridization in 1981. The evidence will be provided by interpretation of references relating to this art and by my personal perspective from having been involved in the field of oligonucleotide chemistry and biology in 1981.

5. **ENABLEMENT**

A. **Analogs of Nucleic Acid**

It is my understanding that the Examiner has concerns that the invention should be limited to phosphotriester-modified nucleic acid and that claims reading on natural nucleic acid and other analogs are not enabled. It is my opinion that once Dr. Tullis identified functional sizes and the mRNA coding region as a target, the invention was fully disclosed to one of skill. The utility of natural nucleic acid and various analogs to be internalized by cells and inhibit cell function was known. Evidence of these facts can be found in the prior art. For example, Miller (1977) describes neutral, nonionic nucleic acids for nonspecific inhibition of protein synthesis. Before (1974) used methylated ribonucleic acid to inhibit viral replication. Zamecnik and Stephenson (1978) used a natural phosphodiester DNA tridecamer to inhibit viral replication *in vivo*. The DNA was analyzed for purity by me. Finally, Summerton (1979) described a number of early reports using various nucleic acid analogs to inhibit viral infections (see page 89).

B. **Making Ribonucleic Acid**

The Examiner further argues that there is no teaching of how to make or use ribonucleotides. The methodology for making synthetic RNA and its analogs was available in 1981. Evidence of this fact can be found in the Miller reference (1977). Therein the authors used an analog of ribonucleic acid to non-specifically inhibit protein synthesis.

Moreover, methods for chemical synthesis of oligoribonucleotides were well established by 1981 as is illustrated by reference to the work of Ohtsuka and his colleagues. In the mid and late 1970s, Ohtsuka's group reported synthesis of numerous oligoribonucleotides corresponding to the sequence of an *E. coli* tRNA. Some of this work is described in Nuc. Acids. Res. Symp. Series (NARS) No. 7, pp. 335-343 (1980), which is attached as Exhibit 3, and the cites therein. In 1980 Ohtsuka reported synthesis of oligonucleotides corresponding to the total sequence of *Escherichia coli* $tRNA_f^{met}$; these oligos were joined using RNA ligase to create an entirely synthetic tRNA (*Id.*). The synthesis of *E. coli* tRNA is also discussed in a subsequent paper that appeared prior to the filing date of the application [Proc. Nat. Acad. Sci. 78(9) 5493 (1981); attached as Exhibit 4]. Clearly, by 1981 methods for chemical synthesis had been available for several years.

Enzymatic methods for synthesis of oligoribonucleotides complement chemical methods and include use of polynucleotide phosphorylase and T4 RNA ligase. RNA ligase in particular has been useful in synthesis of oligoribonucleotides and in 1980 Gumport *et al.*, in a paper on T4 RNA ligase, observed that "...the enzyme is now widely used to synthesize defined sequences of RNA." [NARS No. 7 (1980) pp. 167-171 at 167; attached as Exhibit 5]. In a 1981 review attached as Exhibit 6, ("T4 RNA Ligase as a Nucleic Acid Synthesis and Modification Reagent" in *Gene Amplification and Analysis*, Vol. 2, Chirikjian and Papas, eds. Elsevier (New York) 1981, pp. 314-345 at pages 335-339), Gumport and Uhlenbeck describe work by several groups engaged in oligoribonucleotide synthesis, including Ohtsuka [described above], Neilson and colleagues [using a combination of organic and enzymatic methods to prepare several decanucleotides], Krug and colleagues [preparation of a 21-nucleotide RNA], and others [see citations at 337, first full paragraph]. In my opinion, the attached exhibits clearly demonstrate that methods for synthesis of oligoribonucleotides were well known in 1981.

C. Cell Uptake of Nucleic Acid

Finally the Examiner raised the issue of cell uptake of nucleic acid. He comments that there are no data and methods for actually "getting short DNAs or RNAs into cells." Living cells have a natural capacity for internalizing short nucleic acids. There is nothing to teach. The cells internalize these compounds without special culture conditions. There is no need to render the cells porous. The literature relied upon by the Examiner to support his obviousness rejection teaches this fundamental fact. For example, the Miller reference involves the effect of a trinucleotide analog on mammalian cells and the Summerton reference discloses at pages 93-94 the routine uptake by animal cells of both RNA and DNA. Finally there is the paper by Zamecnik and Stephenson (1978) which describes the internalization of viral infected cells by a DNA of 13 nucleotides.

6. OBVIOUSNESS

It is my understanding that the Examiner believes that in 1981 a person of skill reading Itakura *et al.*, Paterson *et al.* or Hastie *et al.* and Summerton or Miller *et al.* would have had a motivation and a reasonable expectation that targeting the coding region of a specific mRNA with a oligonucleotide complementary to the coding region would have arrested protein translation of that mRNA. There are a number of objective reasons why this is not an accurate statement of the state of the art in 1981.

A. The secondary structure of the mRNA made it an unlikely target for control of expression by complementary oligonucleotides.

The claimed invention went against the conventional wisdom of the time. The conventional wisdom in 1981 was that the secondary structure of mRNA was extensive that there was no reasonable likelihood that a oligonucleotide complementary to a coding sequence would have sufficient access to arrest translation inside a living cell. In addition, those of skill understood that the natural mechanism of peptide elongation by ribosomes involved the destabilization of the extensive secondary structure of the mRNA. For these reasons, the idea of hybridizing a complementary oligonucleotide to a coding region of mRNA to arrest translation was contrary to conventional wisdom. The oligonucleotide would have

to overcome two significant hurdles. First it had to bind to the coding region of the mRNA, which was viewed as a Gordian knot of secondary structure. And even if the complementary oligonucleotide could find and anneal to its complementary subsequence, the ribosomes were viewed as able to bind to a specific non-coding site and then read mRNA coding regions constrained by extensive secondary structure. Thus it was not likely that the hybridization of a complementary oligonucleotide would arrest translation. The ribosome would simply "toss" the duplexing structure aside.

Although there is little express language in the prior art articulating the above concerns, the literature indirectly compels one to conclude that secondary structure and ribosomal helix destabilization were concepts teaching away from the invention. References published after the priority date of the subject application do expressly identify the state of the art at the time of the invention.

The extensive secondary structure of mRNA was well understood in 1981. In the book, The Ribonucleic Acids, 1977, Eds. Stewart and Latham, Springer Verlag, Chpt. 4, "Messenger RNA" by J.M. Adams, the extensive secondary structure in phage mRNA and eukaryotic mRNA is described. Those of skill recognized the importance of mRNA secondary structure and particularly were aware of the need of low mRNA secondary structure in the region where ribosomes initially bind to mRNA. W. Salser, in his chapter Globin mRNA Sequences: Analysis of Base Pairing and Evolutionary Implications, in *Chromatin* Vol XLII (1978) pages 985-1001 at page 992, provided a graphic illustration of the extensive amount of secondary structure in the typical mRNA. In Dr. Salser's illustration, the sequences involved in ribosome initiation and interaction are dramatically free of secondary structure. Dr. Adams, on page 103 in his section (e) entitled "Influence of secondary and tertiary structure on initiation," also discusses the importance of secondary structure on initiation of translation. In chapter 7, by Wettenhall and Clarke-Walker of *The Ribonucleic Acids*, the authors on page 258 in a section entitled "Messenger RNA structure" expressly state, "[t]he role of mRNA structure in initiation reactions is receiving considerable

attention." These authors also discuss the role of secondary structure of mRNA on translation efficiency.

The concern over availability of the coding region to bind oligonucleotides is made more apparent when one looks at the secondary references relied upon by the Examiner. These references are Paterson *et al.* and Hastie *et al.* describing cell-free, *in vitro* experiments in which denaturing conditions to relax the secondary structure of their mRNA are applied prior to hybridizing nucleic acids. For example, Patterson used 100°C for 30 seconds and Hastie used temperatures between 45°C and 65°C. For the Examiner to believe that one of skill would have understood that complementary oligonucleotides were able to bind to the coding regions of mRNA under *in vivo* conditions when both Hastie and Paterson used denaturing conditions is logically inconsistent and scientifically incorrect.

In 1980, I was personally working on a project that had similar aspects to Dr. Tullis' work. I was attempting to sequence the RNA genome of Rous Sarcoma Virus. I used a procedure which relied on *in vitro* hybridization of short oligonucleotides to the RSV-RNA, followed by 3' extension of the primers using reverse transcriptase. The resulting cDNA was then sequenced. Like my colleagues seeking to bind DNA to mRNA, the secondary structure of the RSV-RNA was of paramount concern to me. I took a number of deliberate steps to minimize the effect of secondary structure which might block primer attachment and prevent me from obtaining a complete set of cDNA species to sequence. Those steps included heating the viral RNA to 95° in low salt (to remove secondary structure), rapidly chilling the RNA on ice in a low salt buffer (to freeze the RNA in an open conformation), followed by the addition of reverse transcriptase and DNA primers as rapidly as possible (less than one minute), and running the reverse transcription at the highest possible temperature to maintain an open conformation. The work was published in *Cell*, **32**:853-869 (1983), Exhibit 7. I declare that the concerns of secondary structure were known to me in 1981, are evidenced by my work published in 1983, and were of paramount concern to those of skill in the relevant art of the subject invention.

A number of other references taught that the targeting of a coding regions would not be a preferred target for a oligonucleotide agent expected to control expression. Pluskal *et al. Biochem. Soc. Trans. 7*:1091-1093 (1979), wrote that their work with a heterogenous mixture of low molecular weight oligonucleotides non-specifically inhibited translation, but that the mode of action was not affected by preincubation with the mRNA. One of skill is left to conclude that the translation inhibition observed by Pluskal did not occur via complementary binding interactions between the oligonucleotides and the mRNA.

Continuing in temporal order, the Ts'o patent further suggests away from the Applicant's invention. In the Ts'o patent, the claimed oligonucleotide analogues are described as inhibiting expression of a preselected sequence, either a cellular nucleic acid or a viral nucleic acid. Ts'o uses trimer analogues and at column 25, lines 5-10, they state that their analogues inhibited poly(U) messages but not globin messages. In column 26, lines 5-34, Ts'o explains that they saw no inhibition *in vivo* of either bacteria or hamster cells.

Ts'o's results are consistent with later published reports. These later reports actually explain what was intuitively apparent to those of ordinary skill at the time Dr. Tullis filed his application in 1981. There was no objective reason or basis to conclude that the coding region of mRNA was an effective target for *in vivo* control of expression by trinucleotides because there was no evidence that the trimers used by Ts'o inhibited elongation when bound to mRNA either *in vitro* or *in vivo*. Either the coding regions of mRNA were physically unavailable to complementary oligonucleotides or even if binding to the coding regions, the oligonucleotides were unable to arrest the elongation process of the ribosomes. As explained above, the latter concept was particularly compelling because to elongate, ribosomes have to untwist (denature) the secondary structure of mRNA.

Perhaps the most express and compelling articulation of the true state of the art from 1977 until after the effective filing date of the subject application is found in Dr. Miller's own work published in two parallel reports in 1985. In Blake, Murakami and Miller, *Biochemistry 24*, 6132-6138 and *24*, 6139-6145, the authors discuss at length their concerns over secondary structure on the

availability of subsequences of mRNA to complementary oligonucleotides. In the first of the two companion articles (A22), Dr. Miller looked at 8-12 mer oligonucleotides and concluded, on page 6135, that in the cell free rabbit reticulocyte protein expression system, 8-mer oligonucleotides do not arrest protein elongation, but that if bound to the initiation codon region the oligonucleotides will arrest translation. On the second column of page 6136, Dr. Miller in summarizing the state of the art expressly states:

> The above results are in agreement with the recent findings of Liebhaber *et al.* (1984). They found that cDNAs to human globin mRNA which cover the initiation codon or extend into the 5'-noncoding region are able to completely inhibit translation in a rabbit reticulocyte lysate. In contrast cDNAs which cover only the coding region exclusive of the initiation site are not effective at blocking translation. These authors postulate that a helix-destabilizing activity associated with the reticulocyte ribosomes is able to disrupt secondary structure during the elongation step but not the initiation step of translation. **Thus, cDNAs or oligonucleotides bound to the coding region of mRNA would be expected to be unable to prevent translation in the reticulocyte system.** [Emphasis added]

Dr. Miller is summarizing two points to explain why oligonucleotides binding to the initiation codons will inhibit translation but those binding to coding regions will not inhibit translation. He first explains that the ribosomes will simply remove the oligonucleotides bound to the translated region (page 6136, column 2), and second, he explains that the secondary structure of mRNA precludes the binding of the oligonucleotides to the translated region (page 6137). He implies this is true even for cDNAs which may be hundreds of bases long.

In the second article, Dr. Miller is again reporting on the ability of oligonucleotides to arrest *in vitro* translation. In this report the oligonucleotides are between 6 and 11 bases long. The authors are reporting some degree of success with the binding of oligonucleotides to mRNA coding regions. Although the success of the method is being reported, the authors are clearly articulating the concerns over secondary structure which was the conventional wisdom in 1981. For example at page 6144, the authors state:

> In addition to the effect of mRNA secondary structure, the region of the mRNA to which the oligonucleotide methylphosphonates binds influences its effectiveness as an inhibitor of mRNA translation. The results in Table II suggest that oligomers complementary to the 5' end and initiation codon regions are somewhat better inhibitors than the oligomers which bind to the coding regions. **Thus it appears that the initiation step of translation is more sensitive to oligomer mRNA binding than the elongation step of translation.** [Emphasis added]

Finally, as late as 1986, the literature was still suggesting that short oligonucleotides will only arrest translation if bound to the initiation sites. In particular, Haeuptle *et al.,* using a cell free translation system, clearly states that size of the oligonucleotides bound to coding regions is a critical key to ensuring arrest of translation. Just as is taught by the subject application with its effective filing date of 1981, Haeuptle reports in 1986 that the oligonucleotides must be 10 bases or greater in length before substantial inhibition of translation is demonstrated. The Examiner should also note that the authors, in deference to secondary structure, are using 55°C for 5 minutes to relax the mRNA sufficiently to allow the oligonucleotides to bind to their target regions.

In conclusion, I state that the conventional wisdom in the art, at the time of the invention, taught away from Dr. Tullis' invention. The literature cited above is offered as evidence of this wisdom. This literature clearly teaches that the secondary structure of mRNA, especially in the coding region, was thought by those of skill in 1981 to be extensive and that this secondary structure would preclude binding of a complementary oligonucleotide to the coding region mRNA for the purpose of arresting translation. Also, there was reason to believe that the helix destabilization of ribosomes during elongation would have easily displaced any oligonucleotide duplexed on the coding region. Therefore, one of skill would not have considered the claimed invention to have a reasonable expectation of success in 1981 when Dr. Tullis originally filed the subject patent application.

B. <u>The Examiner's interpretation of the text in Miller (1977) goes well beyond the understanding one of skill would have reading the same text in 1981.</u>

It is my further understanding that the Examiner has stated that Miller (1977) expressly states that oligonucleotides complementary to the coding region

of mRNA might inhibit cellular protein synthesis *in vivo*. The two statements have been cited by the Examiner. The abstract states:

> Our biochemical studies suggest that inhibition of cellular protein synthesis might be expected if $G^mp(Et)G^mp(Et)U$, $G^mp(Et)G^mpU$, and G^mpG^mpU, which have been taken up by or formed within the cell, physically bind to tRNA and mRNA and inhibit the function of these nucleic acids.

The last paragraph states:

> The triester $G^mp(Et)G^mp(Et)U$ is complementary to target nucleic acids involved in cellular protein synthesis and has a transitory effect on the cells which is then relieved by its degradation. Such a transitory effect of inhibition may provide a convenient and useful way of imposing a temporary interruption of cellular functions. On the other hand, neutral oligonucleotide analogues with longer chain lengths and a greater resistance to hydrolysis would be expected to achieve a greater specificity and effectiveness in regulating the function of target nucleic acids inside the cell over a long period of time. The observations of the present study lay the groundwork for continued investigation into the use of neutral oligonucleotide analogues as proves and regulators of nucleic acid function within living cells.

There are multiple reasons why one of skill in 1981 would not have interpreted the cited text in the way the Examiner suggests. The text clearly does not state that oligonucleotide analogs could bind to the coding regions of mRNA. At best it is ambiguous when taken out of context both literally and historically. It is literally ambiguous because the authors' work is directed to general inhibition of all protein expression by using a trimer to bind to the amino acid accepting codon of a tRNA. There is no evidence of binding to target mRNA or of any inhibition of specific protein expression due to complementary binding of Miller's trimer to mRNA.

The phrase "greater specificity" is patently ambiguous. Statistically, any trimer sequence is represented every 64 nucleotides and therefore a trimer binds nonspecifically. Miller's use of "greater specificity" could refer to oligonucleotides that are specific (only) for the amino acid accepting codon of tRNA or that bind specifically (only) to the initiation region of a mRNA, or thirdly, as the Examiner would read the passage, using longer oligonucleotides that would bind with greater specificity to the coding region of an mRNA.

In historical context, the meaning of the above quoted text is clearly directed to either the tRNA or mRNA binding sites. These were only regions perceived by scientists in 1981 as being sufficiently accessible to complementary oligonucleotide binding under *in vivo* conditions. As explained above, the extensive secondary structure of mRNA was a primary reason why those of skill would have avoided the coding region of mRNA to arrest translation. For example, in Dr. Salser's review article of 1978, he includes a figure (Figure 3) depicting the proposed secondary structure of a mRNA. It is an extraordinary complex and twisted structure. Only a few regions are accessible to a complementary oligonucleotide and these are designated as points of initiation and tRNA interactions. These open regions which are generally found as single-strand hairpin loops.

Furthermore, the historical record indicates that by April of 1981, Dr. Mill understood the limitations of his 1977 report. In *Biochemistry*, **20**:1874-1880, Exhibit 8, Dr. Miller *et al.* reported on the arrest of globin expression via blockage of tRNA aminoacylation by trimers and tetramers. On page 1879, and at Table VI, they report no effect on globin synthesis despite the fact that the oligonucleotides used by Miller (polyA) could bind to at least three sites in the coding region of mRNA encoding globin (codons 45, 85 and 118). See Exhibit 9.

Finally, in 1985, the last two Miller references summarize the historical evidence that taught away from targeting the coding regions of mRNA with oligonucleotide to arrest translation. First, in the introductions to both 1985 papers, there is no reference to the 1977 paper when discussing mRNA as a target for control of protein expression by complementary oligonucleotides. Secondly, as stated above on pages 7-8, the 1985 references specifically suggest; (1) that secondary structure will prevent binding of oligonucleotides to mRNA; and, (2) that helix destabilizing properties of ribosomes will remove oligonucleotide even it they were to have access to the coding region of an mRNA. These are two conclusive reasons why one of skill would not expect the arrest of protein translation by oligonucleotide binding to the two conclusive coding regions of mRNA.

In summary, by 1981, Miller's reference to "greater specificity" would not have been interpreted as a suggestion for controlling the expression of particular "target" proteins by binding to specific coding regions of mRNA, but as a suggestion to use longer oligonucleotides to bind **specifically** to the open regions of the tRNA or mRNA that bind to rRNA during the initiation step of protein synthesis. The open binding sites are longer than three bases and thus one would expect greater specificity for binding by using oligonucleotides greater than three bases. For these reasons, I state definitively that one of skill reading Miller in 1981 would have recognized that the proposed targets for binding oligonucleotides are these binding sites.

To argue that in 1981, one of ordinary skill would have predicted *in vivo* utility for an oligonucleotide binding to the coding portions of mRNA is to read Miller in a vacuum, and ignores the authors' data and the historical understanding of the accessibility of the coding region of mRNA due to secondary structure.

C. Although the Examiner reads the Miller reference as suggesting the use of an oligonucleotide binding to the coding region of an mRNA, Miller does not suggest this aspect of Dr. Tullis' invention.

The Miller reference is silent as to the target sequences on a mRNA to which its oligonucleotides might bind. Messenger RNAs have several functional domains. The coding region is only one domain. These various domains are described as "signals" by W. Salser in his chapter Globin mRNA Sequences: Analysis of Base Pairing and Evolutionary Implications, in *Chromatin* Vol XLII (1978) pages 985-1001. The signal sequences are described on page 988 as including "ribosome binding, mRNA processing, transport from the nucleus to the cytoplasm and so on".

Dr. Tullis' claims involve only the coding region and one reading Miller would not have been directed to this region. One of skill with knowledge of the secondary constraints of the coding region of mRNA and the mechanism by which ribosomes read mRNA would not have read Miller as suggesting the targeting of the mRNA coding region.

D. Even if the prior art oligonucleotides of Miller were binding to the coding region of an mRNA, they could not have arrested translation.

As further evidence that the Examiner's interpretation of Miller goes beyond any reasonable interpretation of the reference by one of skill in 1981, I would like to point out that the inhibition detected by Miller was in fact solely due to a non-specific interference of tRNA. We know this because Miller uses only trimer oligonucleotides and trimers were later shown to be useless as inhibitors of translation even if bound to a coding region of an mRNA.

The Examiner is directed to the 1986 reference of Haeuptle *et al.* Haeuptle describes the *in vitro* arrest of translation by oligodeoxyribonucleotides. The authors relaxed the secondary structure of mRNA encoding lysozyme using 55°C and hybridized oligonucleotides of varying lengths to a portion of the coding region. On page 1435 and in Figure 4 on page 1439, they present evidence of their inability to arrest translation using short oligonucleotides of 5 bases. The 5-mer species are simply too small to have sufficient binding strength to arrest the progress of a ribosome. The authors reported successful arrest of translation with their 10, 15 and 20 mer species.

E. The Examiner has misinterpreted the use of "specificity" by Miller in 1977.

The Examiner relies on the final paragraph of Miller, 1977 stating: "... oligonucleotide analogs with longer chain lengths and a greater resistance to hydrolysis would be expected to achieve a greater *specificity* and effectiveness in regulating the function of target nucleic acids...". The Examiner reads this sentence to include a suggestion to use oligonucleotides for longer binding to the coding regions of mRNA. Above I have explained that this phrase is and would have been understood by one of skill in 1981 to be directed to the non-coding regions of mRNA. I would like to focus this part of my declaration on evidence that the authors' reference to "specificity" was in a general context and did not refer to the coding regions of mRNA encoding specific proteins. More particularly, the authors were referring to oligonucleotides that bound more specifically to the amino acid accepting end of the tRNA and the initiation sites of mRNA.

Dr. Miller's later publication, Blake *et al.* (1985A) provides evidence of his intended meaning in using "specificity" in 1977. On page 6137, column 2 is the word "specific[1]" used in the same context that the word "specificity[2]" was used in 1977. Miller is again asserting that his work suggests *specific* control of expression by binding oligonucleotides to mRNA. But the context of the 1985 reference clearly implies that the arresting oligonucleotides are not directed to the coding regions of the mRNA. The Blake article states that the only available regions are the initiation points due to secondary structure. Thus the Examiner's interpretation of the text of Miller (1977) in an unlimited and broad context so as to include the coding regions of mRNA is to ignore the scientific realities of the contemporaneous literature.

F. <u>There are objective scientific reasons why one of skill would have not had a reasonable expectation that oligonucleotides complementary to the coding region of an mRNA could arrest translation of a specific protein.</u>

The examiner apparently believes that the cell-free systems of Hastie and Patterson provide one of skill with a reasonable expectation that *in vivo* arrest of protein expression was possible with oligonucleotides. This is not true. Both Hastie and Patterson used cDNAs of lengths that exceed 500 bases. These DNAs required harsh denaturing conditions to effect binding to mRNA. The *in vivo* activity of oligonucleotides which are short DNA species, preferably between 13 to 23 bases, cannot be predicted by the *in vitro* behavior of cDNA. Furthermore, the cDNA used by both Hastie and Patterson are too long to cross a cell membrane. They could not be used for *in vivo* control of translation.

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[1] Dr. Miller states in Blake *et al.* at page 6137, column 2, "The results of this study show that sequence-specific oligodeoxynucleotides can be used to arrest translation of specific mRNAs in a selective manner in cell-free systems."

[2] At page 1995, Miller states: "neutral oligonucleotide analogues with longer chain lengths and a greater resistance to hydrolysis would be expected to achieve a greater specificity and effectiveness in regulating the function of target nucleic acids inside the cell over a long time period."

In addition to the impropriety of comparing cDNA to oligonucleotides, there are a variety of additional objective reasons why there was no reasonable expectation in 1981 that oligonucleotides complementary to the coding region of mRNA could arrest translation of specific proteins *in vivo.* The osmotic potentials, the salt and pH conditions, the microenvironments within the various compartments of the cells, the ability of oligionucleotides to bind divalent cations and affect the electropotential and homeostasis of a cell, the secondary structure of the mRNA *in vivo*, the physical pressure and gel-like consistency of the cytosol, are all factors which precluded the ability of one of skill to predict with a reasonable certainty that complementary oligonucleotides could bind with sufficient strength, if at all, to the coding regions of mRNA to selectively arrest translation. As Dr. W. Salser succinctly stated in his 1978 review article, "The problem is then to assess which parts of the [mRNA] structure in Figure 3 may actually exist in the complex milieu of the cell."

Furthermore, even if one were to ignore the conventional wisdom that the secondary structure of mRNA rendered the coding region inaccessible to complementary oligonucleotides, there were a number of unknown parameters which alone or in combination render the question of successful regulation of expression by the claimed methods unpredictable. More specifically, there were a number of valid reasons why the oligonucleotides expected to arrest translation might not physically reach the mRNA inside a living cell. The mRNA of eukaryotes is produced in membrane bound nuclei. The art demonstrated that oligonucleotides would be taken up by cells, but not whether the oligonucleotides would be allowed access to the internal regions of the nuclei. Before *et al.*, at page 184, states that exogenous oligonucleotides used as antiviral agents enter cells but are primarily bound to the microsomal fractions and do not significantly enter the host cell nuclei. It was known that messenger ribonucleic acid proteins bound to mRNA. According to Pain and Clemens, in *Comprehensive Biochemistry*, Vol 19B, Part 1 at pages 14-15 (1980), the function of these proteins was unknown. These proteins might have played a role in the storage and delivery of the mRNA to the correct ribosomes (membrane bound or not) and might have interfered with the access of

the mRNA complementary oligonucleotides. As the authors go on to explain, only 10% of the total mRNA produced by the cells is actually used by the ribosomes to produce protein. That unknown mechanism, perhaps controlled by messenger ribonucleoprotein, as well as other unpredictable aspects of mRNA transport, could very well have rendered the mRNA unaccessible to complementary oligonucleotides.

In addition, there were the unknown effects of spermidine and spermine. These common polyamines tightly bind to the phosphate backbone of mRNA *in vivo* and play a role in structure and function of mRNA and ribosomes during protein synthesis. The impact of spermidine on the ability of oligonucleotides to bind *in vivo* to mRNA, either because the polyamines binding to the oligonucleotides might prevent hybridization to mRNA, or because spermidine bound to mRNA might block oligonucleotide binding *in vivo*, was simply unknown. Thus, the effect of spermidine and spermine was yet another unknown factor that would lead one of skill away from a reasonable expectation that one could effect the *in vivo* arrest of translation by oligonucleotides complementary to the coding region of specific mRNA.

In conclusion, it is clear that one of skill would not have expected, with any reasonable degree of certainty, in 1981 that an oligonucleotide specific for the coding region of an mRNA could arrest translation. In summary, the following seven objective reasons were identified above: (a) that the intact mRNA might not be physically accessible to complementary oligonucleotides; (b) that secondary structure of mRNA might block complementary oligonucleotide binding; (c) that short oligonucleotides might not have sufficient binding strength to block a ribosome designed to untangling internal duplexes in mRNA; (d) that the use of unsuitably long complementary oligonucleotides would have their own secondary structure that would interfere with hybridization of mRNA; (e) that polyamines and transport proteins might have rendered the coding region of mRNA inaccessible; (f) that the ability of oligonucleotides to bind cations might have had a toxic affect in target cells; and (g) that the majority of mRNA are not actually translated by cells but rapidly turned over--the flooding of a living cell with oligonucleotides at the

concentrations necessary to effectively bind to mRNA might have been toxic in a non-specific way.

This Declarant has nothing further to say.

Dated: August 17, 1994

Dennis E. Schwartz

Dennis E. Schwartz, Ph.D.

attachments:

Exhibit 1 [Ruth C.V.]
Exhibit 2 [Schwartz C.V.]
Exhibit 3 [Ohtsuka *et al.* 1980]
Exhibit 4 [Ohtsuka *et al.* 1981]
Exhibit 5 [Gumport *et al.* 1980]
Exhibit 6 [Gumport and Uhlenbeck 1981]
Exhibit 7 [Schwartz *et al.* 1983]
Exhibit 8 [Miller *et al.* 1981]
Exhibit 9 [Efstratiadis *et al.* 1977]

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Att #29

Richard H. Tullis
Serial No.: 08/078,767
EXHIBIT 1

Jerry L. Ruth, Ph.D.

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Ashland, OR 97520
Phone: (503) 482-4834 home
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Summary of Professional Qualifications

A skilled scientist and laboratory director with fifteen years experience in diagnostic and therapeutic research laboratories, four years teaching and research experience in academic labs, and three years experience in forensic labs. Advanced skills and training in organic chemistry, nucleic acid chemistry, biochemistry, molecular biology, pharmacology/toxicology, forensics, and clinical chemistry. Ten years laboratory management and supervision in the biomedical field. Experienced in intellectual property rights and corporate safety management. Primary area of research interest is nucleic acid chemistry, and its application to genetics, diagnostics and therapeutics.

Education

Ph.D., Synthetic Organic Chemistry (biochemistry minor) 1978
University of California, Davis, California

B.Sc., *cum laude*, Chemistry 1974
Southern Oregon State College, Ashland, Oregon

Experience

U.S. Fish and Wildlife Forensics Lab 1991-present

Senior Forensic Scientist, DNA Analysis

- DNA analysis, specializing in DNA fingerprinting
- research in methods for identification of individuals and populations of wildlife, including threatened and endangered species
- forensic wildlife casework for State, Federal, and foreign law enforcement agencies

Molecular Biosystems, Inc., San Diego, California 1982-1991

Vice President, Research, Principal Scientist, 1986-1991

- basic and applied research in DNA amplification, solution detection, modified oligonucleotide chemistry, and clinical automation
- management of thirty laboratory people and a $2.5 million budget
- corporate patent and intellectual property protection
- identification of business opportunities, strategic planning and implementation of new medical diagnostics
- development of FDA-approved diagnostic products, including infectious and genetic diseases, and DNA typing analysis (human and animal)
- corporate safety and hazardous waste management

Director, Research and Development, 1984-1986
- novel methods of fluorescence energy transfer detection
- nonisotopic labeling and detection of synthetic DNA probes
- development of bead-based sandwich hybridization assays
- federal grant funding and research grant management

Director, Nucleic Acids Chemistry, 1983
- new diagnostic assay formats, including dot blot, sandwich, and *in situ*
- synthesis and characterization of modified and labeled oligonucleotides

Research Scientist, 1982
- design and synthesis of topical pharmaceuticals
- transdermal delivery of antipsoriatic drugs

University of North Carolina, Chapel Hill 1980-1982

Department of Pharmacology and the Cancer Center
NIH Young Investigator's Award and Staff Research Associate
- molecular pharmacology of viral and cancer drugs
- mechanism of human herpes virus' infections and treatments
- design and synthesis of antiviral and anticancer nucleosides/tides
- staff teaching in pharmacology and biochemistry

Calbiochem-Behring Corporation, La Jolla, CA 1978-1980

Senior Research Chemist, 1979-1980
- supervision of bio-organic research program, nucleic acids

Research Chemist, 1978-1979
- design, synthesis and characterization of nucleotide analogs as research products

American River College, Sacramento, CA 1977-1978
- taught premedical chemistry (nursing program)

University of California, Davis, CA 1974-1978
- Donald E. Bergstrom, graduate advisor
- developed new synthetic methods for modified nucleosides
- taught organic chemistry labs and lectures to chemistry majors

Grants and Fellowships (includes only grants where listed as Principal Investigator)

National Cancer Institute Grants (3), 1984,1986-1987
National Institute of Allergy & Infectious Diseases Grants (3), 1984,1985-1986
NIH National Research Service Award, 1981-1983
UNC Cancer Research Center Training Grant fellowship, 1980-1981
UCD Chancellor's Patent Fund Grant, 1976-1977

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Available upon Request

Publications, Refereed Journals

D.E. Bergstrom and J.L. Ruth, "Synthesis of C-5 Substituted Pyrimidine Nucleosides *via* Organopalladium Intermediates", *J. Amer. Chem. Soc.* 98:1587-1589 (1976).

D.E. Bergstrom and J.L. Ruth, "Preparation of C-5 Mercurated Pyrimidine Nucleosides", *J. Carb. Nucleosides Nucleotides* 4:257-269 (1977).

J. L. Ruth and D.E. Bergstrom, "C-5 Substituted Pyrimidine Nucleosides. 1. Synthesis of C-5 Allyl, Propyl, and Propenyl Uracil and Cytosine Nucleosides *via* Organopalladium Intermediates", *J. Org. Chem.* 43:2870-2876 (1978).

Y. Wataya, A. Matsuda, D.V. Santi, D.E. Bergstrom, and J.L. Ruth, "*Trans*-5-(3,3,3-trifluoro-1-propenyl-2-deoxyuridylate: A Mechanism-Based Inhibitor of Thymidylate Synthetase", *J. Med. Chem.* 22:339-340 (1979).

Y.C. Cheng, S. Grill, J.L. Ruth, and D.E. Bergstrom, "Anti-Herpes Simplex Virus and Anti-Human Cell Growth Activity of *E*-5-Propenyl-2'-deoxyuridine and the Concept of Selective Protection in Antivirus Chemotherapy", *Antimicrob. Agents Chemother.* 18:957-961 (1980).

D.E. Bergstrom, J.L. Ruth, and P. Warwick, "C-5-Substituted Pyrimidine Nucleosides. 3. Reaction of Allylic Chlorides, Alcohols, and Acetates with Pyrimidine Nucleoside-Derived Organopalladium Intermediates", *J. Org. Chem.* 46:1432-1441 (1981).

J.L. Ruth and Y.C. Cheng, "Nucleoside Analogues with Clinical Potential in Antivirus Chemotherapy: the Effect of Several Thymidine and 2'-Deoxycytidine Analogue 5'-Triphosphates on Purified Human (α,β) and Herpes Simplex Virus (Types 1,2) DNA Polymerases", *Mol. Pharm.* 20:415-422 (1981).

J.L. Ruth, S.K. White and D.E. Bergstrom, "Direct Preparation of ^{14}C-Labeled 5-Allyl-and 5-Propyl-2'-deoxyuridine from [2-^{14}C] 2'-deoxyuridine.", *J. Labeled Cmpds. Radiopharm.* 19:861-866 (1982).

J.L. Ruth, and Y.C. Cheng, "Selective Antiviral Agents. The Metabolism of 5-Propyl-2'-deoxyuridine and Effects on DNA Synthesis in Herpes Simplex Type 1 Infections.", *J. Biol. Chem.* 257:10261-10266 (1982).

K. Nakayama, J.L. Ruth, and Y.C. Cheng, "Differential Effect of Nucleoside Analog Triphosphates on Ribonucleotide Reductases from Uninfected and Herpes Simplex Virus (Type 1 and Type 2)-Infected HeLa Cells", *J. Virology* 43:325-327 (1982).

Y.C. Cheng, R.S. Tan, J.L. Ruth and G. Dutschman, "Cytotoxicity of 2'-Fluoro-5-iodo-1-β-D-arabinofuranosylcytosine and Its Relationship to Deoxycytidine Deaminase.", *Biochem. Pharmacol.* 32:726-729 (1983).

D.E. Bergstrom, J.L. Ruth, P.A. Reddy, and E. DeClercq, "Synthesis of *E*-5-(3,3,3-Trifluoro-1-propenyl)-2'-deoxyuridine and Related Analogues: Potent and Unusually Selective Antiviral Activity Against Herpes Simplex Virus Type 1.", *J. Med. Chem.* 27:279-284 (1984).

E. Peterson, S. Aarnaes, R. Bryan, J.L. Ruth, and L. de la Maza, "Typing of Herpes Simplex Virus with Synthetic DNA Probes.", *J. Infect. Dis.* 153:757-762 (1986).

E. Jablonski, E. Moomaw, R. Tullis, and J.L. Ruth, "Preparation of Oligodeoxynucleotide-Alkaline Phosphatase Conjugates and Their Use as a Hybridization Probes.", *Nucl. Acids Res.* 14:6115-6128 (1986).

G.L. McLaughlin, J.L. Ruth, E. Jablonski, R. Steketee, and G.H. Campbell, "Use of Enzyme-linked Synthetic DNA in Diagnosis of Falciparum Malaria", *The Lancet*, 714-716 (28 March 1987).

J.L. Ruth and E.G. Jablonski, "Synthesis and Hybridization Characteristics of Oligodeoxynucleotide-Alkaline Phosphatase Conjugates", *Nucleosides & Nucleotides*, 6:541-542 (1987).

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G.L. McLaughlin, J.L. Ruth, R. Steketee, and G. Campbell, "Assessment of a Synthetic DNA Probe for Plasmodium Falciparum in African Blood Specimens", *Amer. J. Trop. Med. Hygiene* 37:27-36 (1987).

J.A. Brumbaugh, L.R. Middendorf, D.L. Grone, and J.L. Ruth, "Continuous On-line DNA Sequencing Using Oligodeoxynucleotide Primers with Multiple Fluorophores", *Proc. Natl. Acad. Sci.* 85:5610-5614 (1988).

H.A. Rotbart, P.S. Eastman, J.L. Ruth, K.K. Hirata, and M.J. Levin, "Nonisotopic Oligomeric Probes for the Human Enteroviruses", *J. Clinical Micro* 26:2669-2671 (1988).

J.C. Edman, M.E. Evans-Holm, J.E. Marich, and J.L. Ruth, "Rapid DNA Fingerprinting Using Alkaline Phosphatase-Conjugated Oligonucleotides", *Nucl. Acids Res.* 16:6235 (1988).

L.R. Middendorf, J.A. Brumbaugh, D.L. Grone, C.A. Morgan, and J.L. Ruth, "Large Scale DNA Sequencing", *Amer. Biotech. Lab.*, August, p14-22 (1988).

Y. Ichimiya, P. Emson, C. Christodoulou, M.J. Gait, and J.L. Ruth, "Simultaneous Visualization of Vasopressin and Oxytocin mRNA-Containing Neurons in the Hypothalamus Using Nonradioactive In-situ Hybridization Histochemistry", *J. Neuroendocrinology* 1:73-75 (1989).

F. Baldino, J.L. Ruth, and L.G. Davis, "Nonradioactive Detection of Vasopressin mRNA with *in situ* Hybridization Histochemistry", *Exptl. Neurobiol.* 104:200-207 (1989).

H. Kiyama, P.C. Emson, and J.L. Ruth, "Distribution of Tyrosine Hydroxylase mRNA in the Rat Central Nervous System Visualized by Alkaline Phosphatase *in situ* Hybridization Histochemistry", *Eur. J. Neuroscience.* 2:512-524 (1990).

S.J. Augood, J.L. Ruth, and P.C. Emson, "A Rapid Method of Non-radioactive Northern Blot Analysis", *Nucl. Acids Res.* 18:4291 (1990).

J.L. Ruth, "Forensic DNA Analysis in Widlife Law Enforcement", *Clincal Chemistry* 40 (1994).

Patents and Patent Applications (US and European only)

"C-5 Substitutes Uracil Nucleosides." D.E. Bergstrom and J.L. Ruth, U.S. Patent Number 4,267,544, issued 27 January, 1981.

"C-5 Substituted Cytosine Nucleosides." D.E. Bergstrom and J.L. Ruth, U.S. Patent Number 4,267,171, issued 12 May, 1981.

"Single-stranded Labelled Oligonucleotides, Reactive Monomers, and Methods of Synthesis", J.L. Ruth, US Patent Number 4,948,882, 14 August 1990.

"Defined Sequence Single-strand Oligonucleotides Incorporating Reporter Groups, Process for the Chemical Synthesis Thereof, and Nucleosides Useful in Such Syntheses", J.L. Ruth, European Patent Specification Number 0 135 587, 2 May 1990.

"Process and Composition for Performing DNA Assays", E. Jablonski, R.Lohrmann, E.Tu, and J.Ruth, pending.

"Circular Extension for Generating Multiple Nucleic Acid Complements", J. L. Ruth and D.A. Driver, pending.

Book Chapters

Y.C. Cheng, J.Y. Chen, D. Derse, G. Dutschman, R. Tan, J.L. Ruth, and S. Caradonna, "Potential Use of Viral Enzymes and Antibodies Against Viral Enzymes in Human Serum in Resolving Clinicoepid miological Problems Associated with Human Herpes Virus-Associated Disease", In Antiviral Chemotherapy: Design of Inhibitors of Viral Function, K.K. Gauri, ed, Academic Press, NY, 1981, pp 13-21.

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J.L. Ruth, "Oligodeoxynucleotides with Reporter Groups Attached to the Base", In Oligonucleotides and Analogues: A Practical Approach, F. Eckstein, ed, Oxford University Press, Oxford, UK, 1991, pp 255-282.

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J.L. Ruth, and J. Marich, "Synthetic Nucleic Acid Probes Conjugated to Enzymes", In Nonradioactive Labeling and Detection of Biomolecules, C. Kessler, ed, Springer-Verlag, Heidelberg, Germany, 1992.

D. Bergstrom, X. Lin, G. Wang, D. Rotstein, P. Beal, K. Norrix, and J.L. Ruth, "C-5 Substituted Nucleosid Analogs", *J. Nucleosides and Nucleotides*, 1-16, 1992.

J.L. Ruth, "Oligonucleotide-Enzyme Conjugates", In Methods In Molecular Biology, Vol 26: Protocols For Oligonucleotide Conjugates, Sudhir Agrawal, ed, Humana Press, New Jersey, 1992, pp 167-185.

J.L. Ruth, "The Individualization of Large North American Mammals", In DNA Fingerprinting: State of the Science, S.D.J. Pena, R. Chakraborty, J.T. Epplen, and A.J. Jeffreys, eds, Birkhauser Verlag, Basel, Switzerland, 1993, pp 429-436.

Invited Talks and Plenary Lectures

"Synthesis of 5-Substituted Pyrimidine Nucleosides", NATO Advance Study Institute on Nucleoside Analogues: Chemistry, Biology, and Medical Applications, Sogesta, Italy, 7-18 May 1979.

"Synthesis and Hybridization Characteristics of Oligodeoxynucleotide-Alkaline Phosphatase Conjugates", 7th International Round Table on Nucleosides, Nucleotides, and Their Biological Applications, Konstanz, Germany, 29 Sept-3 Oct 1986.

"Detection of Viral Infection Using Synthetic Oligonucleotide Probes", 1st Annual Gene Probe Technology (AACC) Meeting, San Diego, CA, Oct 1986.

"Biofluorescent Detection of Oligomer Sandwich Hybridizations", Conference of Therapeutic and Diagnostic Applications of Synthetic Nucleic Acids, Cambridge, UK, 2-4 Sept 1987.

"Design, Synthesis, and Use of Oligonucleotide Probes", 101st Annual Meeting of the Association of Official Analytical Chemists, San Francisco, CA, 14-17 Sept 1987.

Invited Talks and Plenary Lectures, continued:

"Synthetic Nucleic Acid Probes", American Medical Association Conference on DNA Probes in the Practice of Medicine, Washington, DC, 24-26 April 1987.

"The Detection of Viral Disease with Nonradioactive Oligonucleotide Probes", Distinguished Corporate Scientist Lecture Series, State University of New York at Stony Brook, Center for Biotechnology, 11 Jan 1989.

"Detection of Viral Infections Using Enzyme-labeled DNA Probes", The Eleventh Annual Conference on Clinical Laboratory Molecular Analysis, San Diego, CA, 8-10 Feb 1989.

"Non-isotopic Labels for DNA Probes", ASM Workshop, 89th Annual Meeting of the American Society for Microbiology, New Orleans, LA, 13 May 1989.

"Why Synthetic Probes?", Communitech Market Intelligence Conference on DNA Probes: Challenges and Opportunities, San Diego, CA, 13 Nov 1990.

"Forensic Analysis of Wildlife, and the Application of DNA Techniques", Rogue Valley medical Center Perspectives '92, Medford, OR 18 September 1992.

"The Forensic Identification of Individual Deer Using DNA Probes", Northwest Association of Forensic Scientists, Portland, OR, 28 October 1992.

"The Individualization of Large North American Mammals", The Second International Conference on DNA Fingerprinting, Belo Horizonte, Brazil, 11 November 1992.

"Forensic DNA Analysis in Wildlife Law Enforcement", Beyond DNA Probes: The 1993 San Diego Conference on Nucleic Acids, San Diego, CA, 20 November 1993.

Non-refereed Presentations and Publications

R.S. Tan, J.L. Ruth, and Y.C. Cheng, "Substrate Specificity of Human Deoxycytidine Deaminase", *Fed. Proc.*, 1313, abst 6091 (1982).

J.L. Ruth, L.J. Arnold, J.A. Streifel, and R.H. Tullis, "Phosphite Chemistry and Oligonucleotide Synthesis: Reactions of Phosphochloridites with Blocked Nucleosides", 5th International Round Table on Nucleosides, Nucleotides, and Their Biological Applications, Research Triangle Park, NC, 20-22 Oct 1982.

J.L. Ruth, "Chemical Synthesis of Non-radioactively-labeled DNA Hybridization Probes." *DNA*, 3:123 (1984).

J.L. Ruth and R.N. Bryan, "Chemical Synthesis of Modified Oligonucleotides and Their Utility as Non-radioactive Hybridization Probes", *Fed. Proc.*, 2048, abst 3666 (1984).

R. Lohrmann, L.J. Arnold, and J.L. Ruth, "New Solid Supports for DNA Synthesis", *DNA 3:124 (1984).*

J.L. Ruth, L.J. Arnold, and R.N. Bryan, "Chemical Synthesis, Hybridization, and Detection of Oligonucleotides Containing Biotinylated or Fluoresceinated Bases", Nucleic Acid Technologies Foundation Conference on DNA and RNA Probes: Strategies and Applications, Rensselaerville, NY, 6-9 Sept 1984.

L.J. Arnold, R. Lohrmann, and J.L. Ruth, "A Novel Universal Support for DNA and RNA Synthesis", *Fed. Proc.* 43:2048, abst 3669 (1984).

J.L. Ruth, C.A. Morgan, and A. Pasko, "Linker Arm Nucleotide Analogs Useful in Oligonucleotide Synthesis", *DNA* 4:93 (1985).

Non-refereed Presentations and Publications, continued:

J.L. Ruth, R.D. Smith, and R. Lohrmann, "DNA Affinity Supports Using Modified Oligonucleotides Attached to Nylon Membranes", *Fed. Proc.* 44:1622, abst 7088 (1985).

E.M. Peterson, L.M. de la Maza, R.N. Bryan, and J.L. Ruth, "Synthetic DNA Probes for Herpes Simplex Virus (HSV) Detection", Sixth International Congress of Virology, Nov 1984.

M.J. Heller, E.G. Jablonski, J.L. Ruth, and E.C. Hennessy, "Microfluorometric DNA Hybridization Assay System", *Fed. Proc.* 45:1516, abst 205 (1986).

E. Jablonski and J.L. Ruth, "Synthesis of Oligonucleotide-Enzyme Conjugates and Their Use as Hybridization Probes", *DNA* 5:89 (1986).

G.L. McLaughlin, J.L. Ruth, E. Jablonski, R. Steketee, and G.H. Campbell, "Detection of Malaria Parasites Using Enzyme-linked Synthetic DNA Probes", Abstracts of the 87th Annual Meeting of the American Society for Microbiology (ASM), 379, abst C-339 (1987).

J.E. Marich, D.F. Wong, G.D. Chapman, and J.L. Ruth, "Development of a Non-isotopic DNA Probe Test for Salmonella", 2nd Annual Gene Probe Technology (AACC) Meeting, San Diego, CA, 4-6 Nov 1987.

M.J. Heller, E. Hennessy, J.L. Ruth, and E.G. Jablonski, "Fluorescent Energy Transfer Oligonucleotide Probes", *Fed. Proc.* 46:1968, abst 248 (1987).

J.L. Ruth, C.A. Morgan, L.R. Middendorf, D.L. Grone, and J.A. Brumbaugh, "Single-Primer Fluorescent Sequencing", *Fed. Proc.* 46:2018, abst 542 (1987).

D.A. Driver, L.M. Schmidt, C.L. Bridge, E.J. Shrawder, S.M. Freier, and J.L. Ruth, "A Rapid Colorimetric DNA Probe Test for Rotavirus in Stool", 2nd Annual Gene Probe technology (AACC) Meeting, San Diego, CA, 4-6 Nov 1987.

L.A. Risen, S.M. Freier, L.L. Menees, and J.L. Ruth, "Nonradioactive DNA Probe-based Technology Compared to Standard ELISA and Bioassay Methods for the Detection of Enterotoxigenic E. coli", 2nd Annual Gene Probe Technology (AACC) Meeting, San Diego, CA, 4-6 Nov 1987.

J.E. Marich, J.L. Ruth, C.J.T.F. Whetstone, W.E. Collins, and G.L. McLaughlin, "Specific Non-isotopic Detection of *Plasmodium falciparum* in Blood Smears Using Enzyme-linked Synthetic DNA", ASTMH National Meeting, 1988.

R.G. Krause, F. Baldino, J.L. Ruth, and L.G. Davis, "*In situ* Hybridization Histochemistry Using Enzyme-Labeled Synthetic Oligonucleotide Probes", Society for Neuroscience National Meeting, Toronto, Canada, 13-18 Nov 1988.

E.J. Shrawder, L.A. Risen, D.B. Roszak, J.E. Marich, and J.L. Ruth, "Detection of Campylobacter by Colony Hybridization with Colorimetric Oligonucleotide Probes", *Clinical Chemistry* 34:1176, abst 113 (1988).

J.H. Kerschner, P.S. Eastman, K.K. Hirata, J.E. Marich, and J.L. Ruth, "Colorimetric Detection of Cytomegalovirus by *in situ* Hybridization Using Enzyme-labeled DNA Probes", Abstracts of the 88th Annual Meeting of the American Society for Microbiology (ASM), abst S-34 (1988).

H. Kiyama, J.L. Ruth, and P.C. Emson, "The Detection of Somatostatin Gene Expression by On-isotopic *in situ* Hybridization Histochemistry", 12th Annual Meeting of the European Neuroscience Association, Turin, 6-8 Sept 1989.

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EXHIBIT 2

CURRICULUM VITAE

Dennis E. Schwartz, Ph.D.

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Origen Corporation

Birth Date and Place: July 25, 1945
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Education:

1972 Ph.D. in Chemistry and Biological Sciences
Interdepartmental Program
Purdue University
Research Director: Dr. Peter Gilham

1967 B.A. in Chemistry
Western Washington State University

Postgraduate Training:

1977-1983 Postdoctoral Training with Dr. Walter Gilbert
(Nobel Laureate in Chemistry, 1981; Member of the National Academy of Sciences) Harvard University

1973-1977 Postdoctoral Training with Dr. Paul Zamecnik
(National Medal of Science, 1991; Member of the National Academy of Sciences) Harvard Medical School

Academic and Professional Appointments:

1993-Present Founder
Origen Corporation
Redmond, Washington

1991-1993 Assistant Professor
Department of Experimental Pediatrics
University of Texas MD Anderson Cancer Center
Houston, Texas

1989-1991 Visiting Scientist
Virginia Mason Research Center
Seattle, Washington

1986-1989	Director of Research and Co-Founder MicroProbe Corporation Bothell, Washington
1983-1985	Program Manager in Biochemistry Genetic Systems Corporation Seattle, Washington
1977-1983	Research Fellow in Biology Harvard University Cambridge, Massachusetts
1977-1983	Member of Board of Tutors in Biochemical Sciences Harvard University Cambridge, Massachusetts
1976-1977	Instructor in Medicine Harvard Medical School Boston, Massachusetts
1973-1976	Research Fellow in Medicine Harvard University Boston, Massachusetts
1967-1972	National Science Foundation Biophysics Traineeship Purdue University W. Lafayette, Indiana

Committee Memberships:

a. National and international:

1987 Small Business Innovation Research (SBIR) Grant Review Committee
National Cancer Institute

b. Local and state:

1986-1987 DiaTech Program (USAID) for the Development of Diagnostic Tests for the Third World Grant Review Committee, 1986-1987.
Seattle, Washington

Consultantships:

1986-1987	Program for Appropriate Technology in Health (Organization funded by USAID to develop diagnostic tests for Third World diseases) Seattle, Washington
1977-1983	New England Nuclear Research Products Division Billerica, Massachusetts

Honors and Awards:

1977-1983 Member, Board of Tutors in Biochemical Sciences
Harvard University
Cambridge, Massachusetts

Bibliography:

a. Ph D. Thesis: Schwartz, D.E. The Mechanism of Stepwise Chemical Cleavage of Polyribonucleotides: New Methods for RNA Sequence Analysis. Purdue University, 1972.

b. Papers:

Moncla, B.J., Brahm, P. Motley, T.S., Vermeulen, N., Persson, G.R., Page, R.C., Engle, L.D. and Schwartz, D.E. Direct Detection of *Porphyromonas gingivalis* in Sub-Gingival Plaque Samples Using an Oligonucleotide Probe. In process of revision after submission to *J. Clin. Microbiol.*

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Schwartz, D.E., Tizard, R. and Gilbert, W. Nucleotide Sequence of Rous Sarcoma Virus. Cell, 32:853-869, 1983.

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Herr, W., Schwartz, D.E. and Gilbert, W. Isolation and Mapping of CDNA Hybridization Probes Specific for Ecotropic and Non-Ecotropic Murine Leukemia Proviruses. Virology, 125:139-154, 1983.

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Schwartz, D.E., Zamecnik, P.C. and Weith, H.L. Rous Sarcoma Virus is Terminally Redundant: The 3' Sequence. Proc. Natl. Acad. Sci., 74:994-998, 1977.

Schwartz, D.E. and Gilham, P.T. The Sequence Analysis of Polyribonucleotides by Stepwise Chemical Degradation. A Method for the Introduction of Radioactive Label into Nucleotide Fragments after Cleavage. J. Am. Chem. Soc., 94:8921-8922, 1972.

c. Abstracts:

Reid, M.W., Schwartz, D.E., Scholler, J. and Hmrichsen, R.D. Nuclear Targeting of Peptide-Oligodeoxynucleotide Conjugates. Accepted for the American Chemical Society National Meeting at San Diego, CA, March 1994.

Vermeulen, N., Adams, D., Dix, K., Kanemoto, R., Layton, T., Motley, T., Petrie, C., Smith, T., Van Ness, J. and Schwartz, D. Detection of Periodontal Bacteria with Oligonucleotide Probes. 89th Annual Meeting of the American Society for Microbiology at New Orleans, LA, May, 1989.

Sheiness, D., Watanabe, S., McArdle, S., Lee, D., Dix, K. and Schwartz, D. Bacterial Vaginosis: Detection with Oligonucleotide Probes. 89th Annual Meeting of the American Society for Microbiology at New Orleans, LA, May, 1989.

Schwartz, D.E. DNA Probes for the Detection of Periodontal Pathogens. The 18th Annual Session of the American Association for Dental Research at San Francisco, CA, March, 1989.

Moncla, B.J., Schwartz, D.E. and Braham, P. Oligonucleotide Probes for Periodontopathogens. The 18th Annual Session of the American Association for Dental Research at San Francisco, CA, March, 1989.

Dix, K., Adams, D.A., Lagrange, A., Petrie, C.R., Scholler, J., Sheiness, D. and Schwartz, D. Synthetic Antisense Oligonucleotides for Trypanosome Chemotherapy. Parasites: Molecular Biology, Drug and Vaccine Design at Keystone, CO, April, 1989. (18th Annual Meeting of the UCLA Symposia on Molecular and Cellular Biology).

Dix, K., Lee, D., Watanabe, S., Randolph, C., McArdle, S. and Schwartz, D. Detection of *Campylobacter pylori* with DNA probes. Keystone meeting on *Campylobacter pylori*, July, 1987.

Auditore-Hargreaves, K., Mesowitz, F., Schwartz, D. and Noller, A. Appropriate Technologies for the Rapid Diagnosis of Infectious Diseases. Health for All in the Future: A WHO Conference; May, 1987, Lyons, France.

Schwartz, D.E. DNA and RNA Sequencing Techniques. 21st Eastern Analytical Symposium of the American Chemical Society, 1982, New York, NY.

Schwartz, D., Tizard, R., Gilbert, W., Taylor, J. and Guntaka, R. Localization of the Splice Junctions in the Subgenomic mRNAs of RSV. RNA Tumor Virus Meeting, May, 1981, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hunter, E., Bhown, A., Schwartz, D. and Eisenman, R. Organization and Processing of the *gag* Gene Product of RSV. RNA Tumor Virus Meeting, May, 1981, Cold Spring Harbor Laboratory, C ld Spring Harbor, NY.

Hunter, E., Hardwick, M., Davis, G., Brown, A. and Schwartz, D. Organization and Processing of the *env* Gene Product of RSV. RNA Tumor Virus Meeting, May, 1981, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schwartz, D., Tizard, R. and Gilbert, W. Partial Nucleotide Sequence of RSV. RNA Tumor Virus Meeting, May, 1980, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schwartz, D., Tizard, R. and Gilbert, W. The Nucleotide Sequence of Rous Sarcoma Virus. 9th Annual ICN-UCLA Symposia on Molecular and Cellular Biology.

Herr, W., Schwartz, D. and Gilbert, W. Disposition of Ecotropic Proviruses in AKR Mice. 9th Annual ICN-UCLA Symposia on Molecular and Cellular Biology.

Patents Pending and Granted:

Granted:

Schwartz, D.E., Kanemoto, R.H., Watanabe, S.M. and Dix, K. Oligonucleotide Probes for Detection of Periodontal Pathogens. US #5,212,059, 1993.

Schwartz, D.E. The Use of Volume Exclusion Agents for the Enhancement of *in situ* Hybridization. US #4,886,741, 1990.

Thomas, E.K., Schwartz, D.E., Priest, J., Hoffman, A. and Nowinski, R.R. Polymerization-Induced Separation Assay Using Recognition Pairs. US #4,749,647, 1988.

Pending:

Schwartz, D.E. The Use of Ultrasound for the Enhancement of Nucleic Acid Hybridization. US #130,754, 1987.

Schwartz, D.E. and Adams, T. Human Papillomavirus Type Diagnosis with Nucleotide Probes. US #114,559, 1987.

Adams, T.H., Schwartz, D.E., Vermeulen, N., Van Ness, J. and Petrie, C. Methods for Multiple Target Analysis through Nucleic Acid Hybridization. US #388,202, 1988.

Van Ness, J., Vermeulen, N. and Schwartz, D.E.A Universal Procedure for Extraction of Nucleic Acids. US #184,467, 1988.

Adams, T., Schwartz, D.E., Vermeulen, N. and Kanemoto, R. Quantification of Bacteria Using a Nucleic Acid Hybridization Assay. US #378,355, 1989.

Previous Grant and Contract Support:

DNA Probes for the Detection of Human Papillomavirus Types in Man. Dennis E. Schwartz, PI. Phase I Small Business Innovation Research (SBIR) Contract, National Cancer Institute, 09/16/86 to 03/16/87, total award of $49,600.

DNA Probes for the Detection of Periodontal Pathogens. Dennis E. Schwartz, PI. Phase I SBIR Grant, National Institute of Dental Research, 07/01/86 to 12/31/86, total award of $49,734.

A DNA Probe for the Diagnosis of *Campylobacter pylori Gastritis*. Dennis E. Schwartz, PI. Phase I SBIR Grant, National Institute of Diabetes and Digestive and Kidney Diseases, 09/30/86 to 03/31/87, total award of $42,030.

DNA Probes to Detect Human Papillomavirus in Oral Cancer. Dennis E. Schwartz, PI. Phase I SBIR Grant, National Cancer Institute, 09/01/87 to 02/28/88, total award of $48,508.

Synthetic Oligonucleotides for Trypanosome Chemotherapy. Dennis E. Schwartz, PI. Phase I SBIR Grant, National Institute of Allergy and Infectious Diseases, 10/01/87 to 03/31/88, total award of $50,000.

DNA Probes to Detect Predisposition to Type I Diabetes. Dennis E. Schwartz, PI. Phase I SBIR Grant, National Institute of Diabetes and Digestive and Kidney Diseases, 06/01/88 to 11/30/88, total award of $49,910.

DNA Probe for the Diagnosis of Human Papillomavirus Types in Man. Dennis E. Schwartz, PI. Phase II SBIR Contract, National Cancer Institute, 10/19/87 to 04/18/89, total award of $500,000.

DNA Probes for the Detection of Periodontal Pathogens. Dennis E. Schwartz, PI. Phase II SBIR Grant, National Institute of Dental Research, 08/01/88 to 07/31/90, total award of $500,400.

A DNA Probe for the Diagnosis of *Campylobacter pylori Gastritis*. Dennis E. Schwartz, PI. Phase II SBIR Grant, National Institute of Diabetes and Digestive and Kidney Diseases, 09/15/88 to 08/31/90, total award of $476,008.

Synthetic Oligonucleotides for Trypanosome Chemotherapy. Dennis E. Schwartz, PI. Phase II SBIR Grant, National Institute of Allergy and Infectious Diseases, 10/01/89 to 5/30/91, total award of $500,000.

Formal Teaching:

Courses taught:

1967-1968	General Chemistry Teaching Assistant in Chemistry Purdue University
1970-1971	Techniques in Biochemistry Teaching Assistant in Biological Sciences Purdue University
1977-1983	Readings in Molecular Biology for Biochemical Science Majors Board of Tutors at Harvard University

Supervisory Teaching:

a. Supervisory committees:

Ph.D. Examination Committee for Mercedes Meyer
University of Texas Graduate School
Thesis Advisor: Dr. Ralph B. Arlinghaus

b. Postgraduates supervised:

Overall supervision of 12 Ph.D.s and 20 Research Associates as Director of Research at Microprobe Corporation (1986-1989). Specific supervision on a daily basis of 1 Ph.D. and 2 Research Associates.

Invitations to National or International Conferences:

1989	DNA Probes for the Detection of Periodontal Pathogens 18th Annual Session of the American Association for Dental Research, San Francisco, CA.
1986	DNA Probe-Based Tests for the Detection of Infectious Diseases Workshop: Technologies for the Rapid Diagnosis of Infectious Diseases, Washington D.C. (Sponsored by the Program for Appropriate Technology in Health)
1982	DNA and RNA Sequencing Techniques 21st Eastern Analytical Symposium of the American Chemical Society, New York, NY.